

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (Currently Amended) A method for producing an oligosaccharide of interest by a genetically modified cell starting with at least one internalized exogenous precursor, ~~said precursor being involved in the biosynthetic pathway of said oligosaccharide~~, said method comprising ~~the steps of:~~

(i) obtaining a cell that

comprises at least one recombinant gene encoding an enzyme capable of modifying said exogenous precursor or one of the intermediates in the biosynthetic pathway of said oligosaccharide from said exogenous precursor necessary for the synthesis of said oligosaccharide from said precursor, and also the components for expressing said gene in said cell, lacks any enzymatic activity liable to degrade said oligosaccharide, said precursor and said intermediates; and

(ii) culturing said cell in the presence of at least one said exogenous precursor, under conditions enabling the internalization according to a mechanism of passive and/or active transport of said exogenous precursor by said cell and the production of said oligosaccharide by said cell.

2. (Currently Amended) The method as claimed in claim 1, ~~characterized in that wherein~~ said cell also comprises at least one gene encoding an enzyme capable of modifying an endogenous precursor ~~involved in the biosynthetic pathway of said oligosaccharide~~, said enzyme being identical to or different than the enzyme of claim 1 ~~used in the method described above~~, and also to the components for expressing said gene in said cell and ~~characterized in that wherein~~ said cell lacks any enzymatic activity liable to degrade said endogenous precursor.

3. (Currently Amended) The method as claimed in claim 1, wherein ~~characterized in that~~ said cell is a cell ~~chosen from~~ selected from the group consisting of bacteria and yeasts.
4. (Currently Amended) The method as claimed in claim 3, wherein ~~characterized in that~~ the cell is a bacterium, ~~preferably of Escherichia coli type.~~
5. (Currently Amended) The method as claimed in claim 1, ~~characterized in that~~ wherein said modification is ~~chosen from~~ selected from the group consisting of glycosylation, sulfation, acetylation, phosphorylation, succinylation, methylation, and addition of an enolpyruvate group.
6. (Currently Amended) The method as claimed in claim 1, wherein ~~characterized in that~~ said enzyme is an enzyme capable of performing a glycosylation, chosen from glycosyl-transferases.
7. (Currently Amended) The method as claimed in claim 6, ~~characterized in that~~ wherein said enzyme is a glycosyl-transferase ~~chosen from~~ selected from the group consisting of  $\beta$ -1,3-N-acetyl-glucosaminyl-transferase,  $\beta$ -1, 3-galactosyl-transferase,  $\beta$ -1, 3-N-acetyl-galactosaminyl-transferase,  $\beta$ -1, 3-glucuronosyl-transferase,  $\beta$ -1, 3-N-acetyl-galactosaminyl-transferase,  $\beta$ -1, 4-N-acetyl-galactosaminyl-transferase,  $\beta$ -1,4-galactosyl-transferase,  $\alpha$ -1,3-galactosyl-transferase,  $\alpha$ -1, 4-galactosyl-transferase,  $\alpha$ -2, 3-sialyl-transferase,  $\alpha$ -2, 6-sialyl-transferase,  $\alpha$ -2, 8-sialyl-transferase,  $\alpha$ -1, 2-fucosyl-transferase,  $\alpha$ -1, 3-fucosyl-transferase and  $\alpha$ -1, and 4-fucosyl-transferase.
8. (Currently Amended) The method as claimed in claim 1, ~~characterized in that~~ wherein said cell culturing is carried out on a carbon-based substrate.

9. (Currently Amended) The method as claimed in claim 8, ~~characterized in that~~ **wherein** said carbon-based substrate is ~~chosen from~~ **selected from the group consisting of** glycerol and glucose.

10. (Currently Amended) The method as claimed in claim 8, ~~characterized in that~~ **wherein** said culturing is performed under conditions allowing the production of a culture with a high cell density.

11. (Currently Amended) The method as claimed in claim 10, ~~characterized in that~~ **wherein** said culturing step comprises:

a) - a first phase of exponential cell growth ensured by said carbon-based substrate,

b) - a second phase of cell growth limited by said carbon-based substrate which is added continuously,

c) - a third phase of slowed cell growth obtained by continuously adding to the culture an amount of said substrate that is less than the amount of substrate added in step b) so as to increase the content of oligosaccharides produced in the high cell density culture.

12. (Currently Amended) The method as claimed in claim 11, ~~characterized in that~~ **wherein** the amount of substrate added continuously to the cell culture during said phase c) is at least 30% less, ~~preferentially 50% and preferably 60% less~~ than the amount of substrate added continuously during said phase b).

13. (Currently Amended) The method as claimed in claim 11, ~~characterized in that~~ **wherein** said precursor is added during phase b).

14. (Currently Amended) The method as claimed in claim 1, ~~characterized in that~~ wherein said precursor is of carbohydrate nature, ~~preferably of oligosaccharide nature.~~

15. (Withdrawn – Currently Amended) The method as claimed in claim 1, wherein ~~characterized in that~~ said precursor is a monosaccharide whose anomeric carbon is linked to an alkyl group so as to allow its internalization by a mechanism of passive transport.

16. (Withdrawn – Currently Amended) The method as claimed in claim 15, wherein ~~characterized in that~~ said alkyl group is an allyl.

17. (Withdrawn – Currently Amended) The method as claimed in claim 15, for the production of ( $\beta$ -D-Gal-[1  $\rightarrow$  4]- $\beta$ -D-GlcNac-1 $\rightarrow$ O-allyl), wherein ~~characterized in that~~

- said cell is a bacterium of *LacZ* genotype;
- said enzyme is  $\beta$ -1, 4-galactosyl-transferase;
- said substrate is glycerol;
- said precursor is allyl-N-acetyl- $\beta$ -D-glucosaminide ( $\beta$ -D-GlcNac-1 $\rightarrow$ O-allyl).

18. (Currently Amended) The method as claimed in claim 1, ~~characterized in that~~ wherein said precursor is lactose.

19. (Currently Amended) The method as claimed in claim 1, ~~characterized in that~~ wherein said precursor is ~~chosen~~ selected from the group ~~composed~~ consisting of:

~~of natural or synthetic  $\beta$ -galactosides, preferably from 4-O- $\beta$ -D-~~  
~~galaetopyranosyl-D-fructofuranose (lactulose), 3-O- $\beta$ -D-~~  
~~galaetopyranosyl-D-arabinose and allyl- $\beta$ -D-galaetopyranoside;~~  
~~of  $\alpha$ -galactosides, preferably melibiose and raffinose, and allyl- $\alpha$ -D-~~  
~~galaetopyranoside; and~~  
~~of sucrose.~~

20. (Currently Amended) The method as claimed in claim 18, ~~characterized in that~~ wherein said ~~active~~ transport of said precursor is performed by lactose permease.

21. (Withdrawn – Currently Amended) The method as claimed in claim 1, wherein ~~characterized in that~~ said precursor is sialic acid.

22. (Withdrawn – Currently Amended) The method as claimed in claim 21, wherein ~~characterized in that~~ said active transport of said precursor is performed by NanT permease.

23. (Withdrawn – Currently Amended) The method as claimed in claim 1, wherein ~~characterized in that~~ said precursor is sialic acid and lactose.

24. (Withdrawn – Currently Amended) The method as claimed in claim 23, wherein ~~characterized in that~~ said active transport of said precursor is performed by lactose permease and NanT permease.

25. (Canceled).

26. (Currently Amended) The method as claimed in claim 25, ~~characterized in that~~ wherein said cell has a ~~genotype chosen from~~ *LacZ* and/or NanA<sup>-</sup> genotype.

27. (Currently Amended) The method as claimed in claim 1, ~~characterized in that it~~ also comprises further comprising the addition of an inducer to said culture medium to induce the expression in said cell of said enzyme and/or of a protein involved in said ~~active~~ transport.

28. (Currently Amended) The method as claimed in claim 27, ~~characterized in that~~ wherein said inducer is isopropyl  $\beta$ -D-thiogalactoside (IPTG) and said protein is lactose permease.

29. (Withdrawn – Currently Amended) The method as claimed in claim 1, for the production of the trisaccharide 4-O-[3-O- (2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl) - $\beta$ -D-galactopyranosyl] -D-glucopyranose, ( $\beta$ -D-GlcNac-[1 $\rightarrow$ 3] - $\beta$ -D-Gal-[1 $\rightarrow$ 4] -D-Glc), **characterized in that wherein:**

- said cell is a bacterium of *LacZ*, *LacY*<sup>+</sup> genotype;
- said enzyme is  $\beta$ -1, 3-N-acetyl-glucosaminyl-transferase;
- said substrate is glycerol;
- said inducer is isopropyl  $\beta$ -D-thiogalactoside (IPTG); **and**
- said precursor is lactose.

30. (Currently Amended) The method as claimed in claim 1, for the production of lacto-N-neo-tetraose and polylactosamine (lacto-N-neo-hexaose, lacto-N-neo-octaose, lacto-N-neo-decaose), **further comprising the addition of an inducer to said culture medium to induce the expression in said cell of said enzyme and/or of a protein involved in said transport-characterized in that wherein:**

- said cell is a bacterium of *LacZ*, *LacY*<sup>+</sup> genotype;
- said enzymes are  $\beta$ -1, 3-N-acetyl-glucosaminyl-transferase and  $\beta$ -1, 4-galactosyl-transferase;
- **said substrate is glucose;**
- said inducer is isopropyl- $\beta$ -D-thiogalactoside (IPTG);
- said precursor is lactose; **and**  
**said culturing occurs in the presence of a glucose precursor,**

31. (Withdrawn – Currently Amended) The method as claimed in claim 30, for the production of a sialyl derivative of lacto-N-neo-tetraose and of polylactosamine (lacto-N-neo-hexaose, lacto-N-neo-octaose, lacto-N-neo-decaose), **characterized in that it also comprises a said further comprising an** enzyme chosen from  $\alpha$ -2, 3-sialyl-transferase and  $\alpha$ -2, 6-sialyl-

transferase, and wherein in that said cell ~~also~~ has a *NanA*<sup>-</sup>, *NanT*<sup>+</sup> genotype and expresses the gene for CMP-NeuAc-synthase.

32. (Withdrawn – Currently Amended) The method as claimed in claim 30, for the production of a fucosyl derivative of lacto-N-neo-tetraose and of polylactosamine (lacto-N-neo-hexaose, lacto-N-neo-octaose, lacto-N-neo-decaose), ~~characterized in that it also comprises a said further comprising an~~ enzyme chosen from  $\alpha$ -1, 2-fucosyl-transferase and  $\alpha$ -1, 3-fucosyl-transferase, and wherein in that said cell ~~also~~ has a *WcaJ* genotype and overexpresses the *RcsA* gene.

33. (Withdrawn – Currently Amended) The method as claimed in claim 30, for the production of a sialyl and fucosyl derivative of lacto-N-neo-tetraose, lacto-N-neo-decaose, ~~characterized in that it also comprises a said further comprising an~~ enzyme chosen from  $\alpha$ -2,3-sialyl-transferase and  $\alpha$ -2,6-sialyl-transferase, and ~~also a said an~~ enzyme chosen from  $\alpha$ -1,2-fucosyl-transferase and  $\alpha$ -1,3-fucosyl-transferase, and wherein in that said cell ~~also~~ has a *NanA*<sup>-</sup>, *NanT*<sup>+</sup>, *WcaJ* genotype and overexpresses the *RcsA* gene and the gene for CMP-NeuAc-synthase.

34. (Withdrawn – Currently Amended) The method as claimed in claim 1, for the production of 3'-sialyllactose ( $\alpha$ -NeuAc-[2→3] -  $\beta$ -D-Gal-[1→4] -  $\beta$ -D-Glc) or 6'-sialyllactose ( $\alpha$ -NeuAc-[2→6] -  $\beta$ -D-Gal-[1→4] -  $\beta$ -D-Glc), ~~characterized in that wherein:~~

- said cell is a bacterium of *LacZ*, *LacY*<sup>+</sup>, *NanA* or *NanT*<sup>+</sup> genotype;
- said enzymes are CMP-NeuAc-synthase and  $\alpha$ -2, 3-sialyl-transferase or  $\alpha$ -2, 6-sialyl-transferase;
- said substrate is glycerol;
- said inducer is isopropyl- $\beta$ -D-thiogalactoside (IPTG); and
- said precursors are lactose and sialic acid.

35. (Withdrawn – Currently Amended) The method as claimed in claim 1, for the production of 3'-fucosyllactose ( $\beta$ -D-Gal-[1 $\rightarrow$ 4]-( $\alpha$ -L-Fuc-[1 $\rightarrow$ 3]-D-Glc) or 2'-fucosyllactose,  $\alpha$ -L-Fuc-[1 $\rightarrow$ 2]- $\beta$ -D-Gal-[1 $\rightarrow$ 4]-D-Glc ~~characterized in that it also comprises a said~~ **further comprising an** enzyme chosen from  $\alpha$ -1, 3-fucosyl-transferase or  $\alpha$ -1, 2-fucosyl-transferase, and ~~wherein in that~~ said cell has a *wcaJ<sup>-</sup> lacZ* genotype and overexpresses the *rcsA* gene and ~~wherein in that~~ said precursor is lactose.

36. (Withdrawn – Currently Amended) The method as claimed in claim 1, for the production of allyl 3-O-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)- $\beta$ -D-galactopyranoside, ( $\beta$ -D-GlcNac-[1 $\rightarrow$ 3]- $\beta$ -D-Gal-1 $\rightarrow$ O-allyl), ~~characterized in that~~ **wherein**:

- said cell is a bacterium of *LacZ<sup>-</sup>, LacY<sup>+</sup>* genotype;
- said enzyme is  $\beta$ -1, 3-N-acetyl-glucosaminyl-transferase;
- said substrate is glycerol;
- said inducer is isopropyl  $\beta$ -D-thiogalactoside (IPTG); **and**
- said precursor is allyl- $\beta$ -D-galactopyranoside.

37. (Withdrawn – Currently Amended) The method as claimed in claim 1, for the production of analogs of lacto-N-neo-tetraose and of polylactosamines in which the glucose residue is replaced with an allyl group, ~~characterized in that~~ **wherein**

- said cell is a bacterium of *LacZ<sup>-</sup>, LacY<sup>+</sup>* genotype;
- said enzymes are  $\beta$ -1,3-N-acetyl-glucosaminyl-transferase and  $\beta$ -1,4-galactosyl-transferase;
- said substrate is glucose;
- said inducer is isopropyl  $\beta$ -D-thiogalactoside (IPTG); **and**
- said precursor is allyl- $\beta$ -D-galactopyranoside.



38. (Withdrawn – Currently Amended) The method as claimed in claim 31, for the production of oligosaccharide analogs in which the glucose residue is replaced with an allyl group, ~~characterized in that~~ wherein said precursor is allyl- $\beta$ -D-galactoside.

39. (Currently Amended) The method as claimed in claim 1, for producing an oligosaccharide labeled with at least one isotope, ~~characterized in that~~ wherein said cell is cultured on said carbon-based substrate labeled with said isotope and/or in the presence of a said precursor labeled with said isotope.

40. (Withdrawn) An oligosaccharide which may be obtained by the method as claimed in claim 1.

41. (Withdrawn) An oligosaccharide which may be obtained by the method as claimed in claim 17, characterized in that the double bond of the allyl group of said oligosaccharides is chemically modified by addition, oxidation or ozonolysis reactions to form activated oligosaccharides that may be used for the chemical synthesis of glycoconjugates or glycopolymers.

42. (Withdrawn) The oligosaccharide as claimed in claim 40, as a medicinal product.

43. (Withdrawn) The oligosaccharide as claimed in claim 42, as a medicinal product intended to selectively prevent the adhesion of biological molecules.

44. (Withdrawn) The oligosaccharide as claimed in claim 42, as a medicinal product intended for treating cancer, inflammation, heart diseases, diabetes, bacterial infections, viral infections and neurological diseases and grafts.

45. (Withdrawn) A pharmaceutical composition, characterized in that it comprises an oligosaccharide as claimed in claim 42 and a pharmaceutically acceptable vehicle.

46. (Withdrawn) The agricultural or agronomic use of an oligosaccharide as claimed in claim 40, especially for the growth and defense of plants.